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## Gene drive: Progress and Prospects

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# Gene drive: Progress and Prospects

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Abstract:

Gene drive is a naturally occurring phenomenon in which selfish genetic elements manipulate gametogenesis and reproduction to increase their own transmission to the next generation. Currently there is great excitement about the potential of harnessing such systems to control major pest and vector populations. If synthetic gene drive systems can be constructed and applied to key species, they may be able to rapidly spread, either modifying or eliminating the targeted populations. This approach has been lauded as a revolutionary and efficient mechanism to control insect-borne diseases and crop pests. Driving endosymbionts have already been deployed to combat the transmission of dengue and Zika virus in mosquitoes. However, there are a variety of barriers to successfully implementing gene drive techniques in wild populations. There is a risk that targeted organisms will rapidly evolve an ability to suppress the synthetic drive system, rendering it ineffective. There are also potential risks of synthetic gene drivers invading non-target species or populations. This Special Feature covers the current state of affairs regarding both natural and synthetic gene drive systems with the aim to identify knowledge gaps. By understanding how natural drive systems spread through populations we may be able to better predict the outcomes of synthetic drive release.

1. Introduction

All organisms harbour a variety of genes that violate the assumption of equal transmission, instead selfishly increasing their frequency in subsequent generations (called drive) at a cost to the genome as a whole. Such selfish genes can make up a substantial proportion of the genome, and show a range of strategies to enhance their spread [1]. Some gene drives are transmission distorters that target gametogenesis to ensure they are over-represented in eggs or sperm following meiosis, resulting in effective transmission distortion. Such meiotic drivers were first described almost a century ago and have been characterised in plants, insects, and mammals [1]. They may be autosomal (e.g. *t* haplotype in house mice *Mus musculus*, which is transmitted from males to up to 100% of offspring – [2]), or linked to one of the sex-chromosomes resulting in sex ratio distortion (e.g. *SR* in flies, causing up to 100% daughters [3]). Synthetic gene drives have recently been developed that produce similar results - transmitting themselves to nearly all offspring. If synthetic gene drive systems can be constructed and inserted into pest populations, they may be able to rapidly spread, potentially disrupting the function of a vital gene leading to population extinction [4, 5], or converting the entire population to males [6]. Alternatively, the gene drive could carry with it a package of genes, aimed at permanently modifying the target population. Possible modifications include making mosquitoes incapable of transmitting malaria [7], or increasing their vulnerability to pesticides.

The potential of harnessing gene drive systems in the control of major pests has been received with both enthusiasm and scepticism. This approach has been lauded as a revolutionary and efficient mechanism to control insect-borne diseases and crop pests, being highly targeted and potentially vastly cheaper than conventional methods such as pesticides [8]. However, there are a variety of barriers, both technical and ethical, to implementing this technique in wild populations. We urgently need to understand how natural drive systems spread through populations if we are to predict the outcomes of synthetic drive release. One

key barrier is the risk that target populations will rapidly evolve an ability to suppress the drive system, rendering it ineffective, as has been seen in natural drive systems [9]. So how much do we currently know about the dynamics of gene drivers?

## 2. Scope of the special issue

This Special Feature issue comprises 14 contributions, covering a wide range of aspects of natural and synthetic gene drivers in a range of animal and plant species. We introduce and discuss these below grouped into three broad topics: (i) Synthetic drive systems, (ii) Natural drive systems, and (iii) Implementation success and wider ethical considerations of gene drives.

### i) Synthetic drive systems

The Special Feature starts with two reviews. The first, Ritchie and Staunton [10], reflects on the lessons to be learnt from 20 years of involvement in the most advanced programme of gene drive intervention: the use of the endosymbiont *Wolbachia* to suppress transmission by mosquitos. They discuss the history of mosquito control, from pesticides, through natural enemies, and sterile male releases, and the limitations of these approaches that have led to the urgent need for more effective solutions. They then discuss the discovery of a strain of the intracellular parasite *Wolbachia*, which when inserted into *Aedes aegypti* mosquitoes reduces dengue transmission to humans. This strain of *Wolbachia* spreads through mosquito populations by cytoplasmic incompatibility: eggs of uninfected females cannot be fertilised in matings with males infected with *Wolbachia*, but eggs of infected females can be fertilised by infected and uninfected males, giving infected females a fitness advantage. Since the release of these mosquitos in Cairns, Australia, the city has been dengue free, making this the most successful gene drive intervention to date.

The second review, by Barrett et al [11], focuses on gene drive in plants; an area where relatively little gene drive work has been carried out. They summarise many of the key opportunities and questions, and discuss strategies to use synthetic gene drive to improve the control of weeds. One key approach is direct population suppression by killing target plant species. However, they suggest that a more useful approach may be modification, making weed species more vulnerable to traditional control techniques such as pesticides. For agricultural uses, this has enormous potential, as it limits the killing effect of the driver to populations targeted by pesticides, radically reducing any impact of the driver on non-target populations. Another interesting use is to enhance the survival of endangered plant species by driving specific useful genes, such as drought tolerance, into vulnerable populations. In particular, the review highlights the issue of seed-banking, the long term persistence of seeds in the soil. Barrett et al [11] show that the seed bank can slow the spread of gene drive by acting as a reservoir of wild type seeds. This issue is largely unique to plants, although it could perhaps be applicable to animals with cryptobiotic phases, such as tardigrades, nematodes, and rotifers.

Beaghton and colleagues [12] focus on gene conversion drive, which have become relatively easy to construct due to the advent of CRISPR/Cas9. This type of drive uses a synthetic

nuclease driver that copies itself onto homologous chromosomes, allowing it to rapidly spread through populations. If the drive disrupts a key gene related to fertility, its spread could radically reduce the productivity of the population. This paper focuses on the issue of non-functional resistance at target genes. Modelling and practical experiments (e.g. Oberhofer et al [13]) have found that the targeted gene can rapidly evolve to be unrecognisable to the driver, preventing gene conversion, and allowing this resistant allele to maintain functional versions of the targeted gene in the population. However, an unappreciated issue is that mutations can also create unrecognisable target alleles without maintaining function in the target gene. Previously this possibility has been largely overlooked, as non-functional resistant alleles still lead to the genes in the population becoming increasingly damaged. However, here Beaghton et al [12] point out that there is typically a cost to the drive mechanism. Non-functional resistant alleles do not bear the cost of drive, and are immune to gene conversion, so can potentially spread through populations containing drive, reducing the spread of the driver. This is a great illustration of how important it is for modelling and empirical work to closely inform each other during the design and use of a synthetic drive.

The next paper, by Holman [14] also models an interesting but unexplored area of gene drive, the potential use of synthetic meiotic drivers in species with ZW sex determination systems. In ZW organisms like butterflies and birds, females have the heterozygous sex chromosomes. The model suggests that W-shredding Z chromosome drivers, whose female carriers only produce sons, should spread extremely rapidly if the evolution of resistance can be avoided. This model is a major step forward in the development of gene drives for unexplored ZW pest species, including the trematodes that cause schistosomiasis, and serious Lepidopteran agricultural pests.

There is also real interest in using synthetic gene drive as a conservation measure to control invasive species such as rats and mice that have caused serious decline to many vulnerable endemic bird, mammal and lizard populations. Godwin and colleagues [15] review the possibilities of using gene drives to control pest populations of rodents. They consider proposed CRISPR- based homology-directed repair drive systems (which have not yet been made to work robustly in mice [16]), and also the modification of a widespread ancient house mouse drive system, the *t* haplotype, into a sex ratio driver. An advantage of co-opting an ancient drive system, in which suppressors have not been found, is that rapid evolution of resistance may be less of a problem. Manser et al. [17] further explore this *t* haplotype based synthetic driver, which is in development. The *t* haplotype is an autosomal sperm-killing driver that manipulates sperm so that nearly all offspring from a heterozygous male inherit the *t* haplotype. The *t-Sry* project aims to take the key mammalian sex determination gene *Sry* from the mouse Y chromosome, and insert it onto the *t* haplotype on chromosome 17, thus creating "*t-Sry*", an autosomal gene drive that turns all individuals that inherit it into a male. The idea is to introduce *t-Sry* to island mouse pest populations, thereby turning the entire population male and eliminating it altogether [18, 19]. Manser et al [17] explore the population dynamics of the *t-Sry* system. They model introductions of *t-Sry* to islands where female mice have varying rates of polyandry (multiple mating). As the *t* haplotype damages sperm, bearers have poor success when the females they mate with also mate with wild type males with undamaged sperm. Manser's models [17] suggest that populations with high rates of polyandry will make it more difficult for *t-Sry* to spread, requiring higher release

effort. As polyandry is widespread in nature [20], these results may be relevant also for other drive systems that reduce male sperm competitiveness.

Godwin et al [15] further highlight some key biological, regulatory and safety challenges for using gene drives in mice. The biology, ecology and behaviour of target island rodent populations remain poorly understood. At least as important, they follow Ritchie and Staunton [10] and George et al. [21] in emphasising how crucial it will be for regulatory frameworks to keep up with the pace of gene drive research, and how vital it is to ensure that the communities and stakeholders affected are consulted, informed, and given a major role in any decisions about the deployment of drives.

## ii) Natural drive systems

Understanding how synthetic drive systems are likely to spread in nature, before any releases, is critical to the assessment of risks and benefits of synthetic drivers. Fortunately, the study of natural drive systems over the last century has provided considerable theoretical and empirical insight into how drivers work and how they spread. Until recently, we have been lacking sufficient data on fitness costs of natural drivers to make models about their spread in nature that match well to driver frequencies observed in wild populations.

In this Special Feature, four studies report on fitness costs associated with male meiotic drivers. These drivers act during sperm development to eliminate their competition, namely, non-driver carrying sperm, which promotes their own transmission. Finnegan et al [22], Larner et al [23], Dyer and Hall [24], and Lea and Unckless [25] measured fitness costs in males and females associated with their species-specific meiotic driver, in stalk-eyed flies *Teleopsis dalmanni*, in the fruit fly *Drosophila pseudoobscura*, in *D. recens*, and in *D. melanogaster*, *D. affinis* and *D. neotestacea*, respectively. These fitness costs are apparent as reduced egg-to-adult viability [22], reduced offspring production in females [23, 24], and reduced sperm competition success [24]. Fitness costs are however trait-specific. Lea and Unckless [25] found no reduced immune function associated with male meiotic drive, and Dyer and Hall [24] found no effects on female mating preferences or on longevity. Larner et al [23] and Dyer and Hall [24] then used the quantified fitness costs to parameterise population genetic models to predict equilibrium frequencies in nature. These predicted frequencies came close to observed frequencies. This is an important step in understanding the dynamics of natural drive systems, and while the details will vary between systems, collectively these studies broaden the appreciation of potential fitness costs in nature. The drive systems investigated in these four studies lie within large chromosomal rearrangements that prevent recombination from breaking up critical drive elements [22, 25]. It remains unknown to what extent these fitness costs arise solely as a consequence of reduced recombination, which allows the accumulation of harmful mutations, or are pleiotropic effects of the drivers themselves.

Fitness costs also select for the evolution of genetic suppressors of drive. Suppressors are present in most *Drosophila* drive systems [26], yet not in *D. pseudoobscura*. Price et al [27] consider why this might be. The low, stable drive frequencies observed in the wild can be explained by fitness costs arising from the combined effect of poor sperm competitive ability of SR males and costs to homozygous SR females. Yet these fitness costs affecting driver

dynamics still imply that the evolution of suppression of drive would be advantageous. The absence of suppressors is therefore puzzling. This *Drosophila* drive system has persisted in nature for at least hundreds of thousands of years [28], leading Price et al [27] to question whether ancient drive systems might be evolutionarily distinct from younger ones. Discovering the mechanisms underlying this drive system would help clarify whether there are particular genetic constraints that make the evolution of suppression less likely, and if they are common to other ancient systems that also have not evolved genetic suppression [27].

Thus an understanding of the genetic architecture of natural drive systems is important for understanding their effects and how drivers evolved, but also can help inform the design of synthetic drivers. Homing endonuclease drive systems were described in yeast and bacteria, later inspiring synthetic homing endonuclease drive systems [4, 29]. The synthetic *Medea* driver developed for the crop pest *Drosophila suzukii* took inspiration from the natural drive system of the same name [30], known from *Tribolium* flour beetles [31]. The development of synthetic X chromosome shredders in mosquitoes [6] is preceded by the discovery in mosquitoes of a natural X chromosome shredder [32], and the synthetic sex ratio distorter being developed in house mice [17-19] is directly based on modification of the *t* haplotype [33]. Courret et al. [26] review the origins and mechanisms of the 19 known drivers in *Drosophila* showing that nearly all of the well-characterised systems evolve from gene duplications and involve heterochromatin regulation, small RNA and/or nuclear transport pathways. Uncovering how these systems work is made difficult by their association with inversions, heterochromatin, and epistatic interactions [26].

Gene expression studies can help identify what elements of drive systems do. In Lindholm et al [34], the transcriptome of the house mouse *t* haplotype, is analysed. Carrying one copy of the *t* haplotype primarily altered the expression in testis of spermatogenesis genes, both of genes mapping to the *t* haplotype but also in a larger number of genes in the rest of the genome. Whether these *trans* gene regulation effects are achieved by transcription factors, non-coding RNA, chromatin modification, or other processes is currently unknown. Other tissues showed fewer differences, and these were mainly localised to the *t* haplotype. This study points to a fine-scaled adaptation of the driver to the rest of the genome, or extensive co-adaptation between them. Can we expect synthetic drivers to evolve to show similar patterns, given enough generations?

iii) Implementation success and wider ethical considerations of gene drive

There has been much discussion of the risks and benefits of harnessing gene drives as a means to regulate and suppress pest and vector populations in the wild – in particular malaria-transmitting mosquitoes [5, 35]. Gene drives have also been proposed as an effective and humane means to regulate invasive species, for example rodents on islands (see Godwin et al [15]; Manser et al [17] in this issue) [36]. The potential benefits are impressive: a reduced risk of insect-transmitted disease, reduced reliance on pesticides with all the associated detrimental side effects (such as bioaccumulation in human food [37], or non-target wildlife poisoning [38]). In addition, there are the increasing costs of pesticide deployment due to the inevitable emergence of resistance, and the continued risk of disease spread by resistant vectors. There are also substantial risks associated with the use of



synthetic gene drives. One risk is the spilling over of gene drives into non-target populations and species. Despite the low likelihood of a gene drive transferring between species, the US National Academy of Sciences currently recommend that the risk of horizontal gene transfer should be evaluated before any environmental release of a gene drive is considered [8]. In addition to the direct risks of gene drives affecting non-target species, it is also important to assess the broader consequences that removal or alteration of the target population or species will have on the wider ecosystem.

The debate surrounding this technology stems in part from insufficient knowledge about natural, let alone synthetic gene drivers. The consensus seems to be that it is not currently possible to evaluate whether the benefits outweigh the risks, but that this should not mean that research and trials using gene drive should be banned. For example, the recommendation by the Royal Society [39] to the UN Convention on Biological Diversity (CBD) is to avoid the adoption of any position that would support an international moratorium on gene drive research, including experimental field trials, a position which was echoed at the United Nations CBD meeting in November last year [40]. The moratorium was eventually rejected. The objection arose in part because if research into gene drives was prohibited, the knock-on effect would be detrimental since it would in effect preclude any wider public debate before we have determined the potential risk and therefore evaluated how we might safely use this technology.

The moratorium was however reworded to emphasise the need to consult with local communities and indigenous groups that are potentially affected before a potential release is considered, echoing the recommendations by George et al [21] and Ritchie and Stanton [10] in this issue. In general, any potential future use of gene drives should be preceded by public debate about the relative appeal of using gene drives compared with alternative solutions. Much importance has been placed on ensuring future research is appropriately governed to encompass a variety of broader societal impacts, in addition to considering biosecurity and unwanted ecological and health impacts [35]. Such a consultative approach is stressed in the contribution by George et al [21], who also highlight the complexities surrounding the ethical considerations of releasing engineered gene drivers in nature. The importance of ensuring sufficient public and political confidence is also emphasised by Ritchie and Staunton [10], who argue that this is key to ensure wider uptake. The success of this approach is exemplified by the work carried out by Target Malaria ([targetmalaria.org/who-we-are/](http://targetmalaria.org/who-we-are/)), a not-for-profit research consortium that aims to develop and share technology for malaria control. The consortium includes scientists, stakeholder engagement teams, risk assessment specialists and regulatory experts from Africa, North America and Europe, and includes an ethics advisory committee.

As yet, apart from making use of naturally occurring endosymbionts such as *Wolbachia* to disrupt disease transmission in mosquitoes, no synthetic gene drive has been released into a wild population. The US Department of Agriculture has excluded genome-edited plants from regulatory oversight, so this may change. The Australian government also recently decided that they will not regulate the use of gene-editing techniques that do not introduce new genetic material into organisms, but will increase their monitoring requirements of gene-drive experiments [41]. In contrast, the European Union Court of Justice has ruled that gene-

edited crops should be treated as genetically modified organisms subject to stringent regulation [42]. Clearly there is no global consensus.

The use of ‘biological’ control measures such as the endosymbiont *Wolbachia* that when introduced into *Aedes aegypti* mosquitoes suppresses transmission of dengue, Zika and chikungunya viruses, has already seen extensive field trials in Australia and elsewhere [10, 43]. The first successful use of cytoplasmically-induced male sterility to control *Culex* mosquitoes was carried out in Burma >50 years ago [44], and several large pilot releases of wMel-modified *Aedes* mosquitoes are currently underway (World Mosquito Program: <http://www.eliminatedengue.com/our-research/wolbachia>). Their successful deployment is reliant on strong community and political support (e.g. the successful World Mosquito Program aimed at eliminating dengue), as without it they are likely to fail, as in the case of several approved trials lacking support [10]. It is noteworthy that the use of naturally occurring agents such as *Wolbachia* (that can cause effective sterilisation by inducing cytoplasmic incompatibility) appears to be less fraught with concerns about their safety compared to synthetic gene drives. However, *Wolbachia*-infected mosquitoes effectively drive genes into populations and can therefore be viewed as analogous to gene drives [45]. Is it possible that the more we learn to harness these naturally occurring gene drivers the more our current apprehension about the use of synthetic drivers will be lessened?

3. Concluding remarks and future directions.

A number of general conclusions and promising avenues for future research emerge from the individual contributions in this issue. Below, we highlight some of the most significant points:

*We need to consider not just the technical but also the ethical and societal aspects of synthetic gene drive.* As Ritchie and Staunton [10], and George et al [21] argue, support from the communities affected by gene drive releases is critical to their successful implementation. It is absolutely essential that any future releases make major efforts to explain all relevant aspects of the project and gain the support of local stakeholders. The furore over genetically modified crops illustrates how badly wrong a project can go if it does not enjoy public confidence. The only way these potentially lifesaving gene drive technologies are going to be practically useful is if they start off well, with successful projects that gain substantial local support. An arrogant top-down approach risks making gene drive technologies politically toxic, rendering them unusable for decades. This would be potentially tragic for human health, agriculture, and conservation. However, there are success stories [10], so this consultative approach can work. Is it possible that there are broad lessons to be learnt from the successful use of harnessing natural systems such as *Wolbachia* to reduce disease transmission in mosquitoes that can be implemented also for deployment of synthetic drive?

*Understanding the costs are key to predicting the dynamics of gene drive.* There have been great inroads made into quantifying the potential costs of gene drive in natural systems as reported in this Special Feature. However, fitness costs can be hard to find: for example, the finding of Finnegan et al [22] of reduced viability associated with meiotic drive in stalk-eyed flies came after multiple previous studies of fitness costs in the same species. In particular,

we need to better document the potential cost of drive in less well characterised natural drive system involving non-model species (i.e. other than flies and house mice). We also do not know if these costs are modified over time as would be predicted by a coevolutionary response. For example, the cost to female *D. simulans* flies of harbouring the Riverside strain of *Wolbachia* has gone from an initial 15-20% fecundity cost to an 10% fecundity advantage after only 20 years of coevolution [46]. Quantifying fitness costs of drive in both males and females is vital to accurately predict the dynamics of drive in natural populations (e.g. [23, 24]). Subtle costs of drive can also affect the success of synthetic drives. Beaghton et al. [12] also investigate the transgenerational impact of empirically demonstrated fitness costs, which has been a surprising discovery in synthetic gene drive research. There is clearly scope both to better refine existing predictive models and to accumulate more data on potential fitness costs in synthetic drive systems to improve our forecasting of synthetic driver dynamics in natural populations.

*The relative importance of balancing costs vs suppression for gene drive success.* Key to the success of implementing synthetic gene drive for population control is their persistence for sufficient amount of time to achieve reduction (or elimination) of the target population. Hence delaying the likelihood and speed of the evolution of suppression is an essential target. However, the persistence of many natural gene drive systems appears to be dependent on the strength of balancing selection [22-24, 27] rather than on the evolution of suppression. Currently we do not know what features of a gene drive system make it more or less likely to be shaped by balancing selection as opposed to suppression. We also do not know if there are any similarities between ancient gene drives in which suppressors have not been found (e.g. sex ratio drive in *D. pseudoobscura*, *t* haplotype in house mice, the long-term persistence of *Wolbachia*-induced male killing in *D. innubila* [47]). In part this lack of insight stems from the limited knowledge about the gene(s) involved in drive, as the mechanisms are not known for many systems [26]. However, just as for sex ratio drive (e.g. *D. simulans*, [9, 48]), there are examples of male-killing systems displaying a dramatic flux of invasion, suppression, replacement, and resurgence of killing across populations (e.g. *Hypolimnas bolina* butterflies [49]). Comparisons between these natural drive systems may reveal potential features that are associated with the long-term persistence of unsuppressed drive systems that could perhaps be incorporated into the design of synthetic drivers. For example, are there potential costly pleiotropic consequences of suppression that are simply too great to overcome? On the other hand, it is possible that long-term persistence of unsuppressed systems is a feature of a complex drive system involving multiple genes and hence is unlikely to be translated in practice to synthetic drivers as they are simply too complex to construct. To date we do not even know if persistent gene drive is associated with a few or many coevolving genes. We clearly need to have a better understanding of the mechanisms of drive and suppression of natural systems before these insights can be translated into the design of synthetic drivers.

In addition, there are several unexplored opportunities of gene drives:

- Many of the proposed uses of gene drives involve reducing harm to humans from disease vectors, humanely removing introduced animals to benefit conservation, or combating crop pests or weeds [11, 15, 35, 36, 50], all of which could reduce deaths from disease and reduce the use of pesticides and poisons. There are, however, other potential uses [11], such as driving beneficial alleles into

populations to rapidly spread adaptive variation. Driving adaptive variation could hasten adaptation to potentially extinction-causing threats, such as climate change, or protecting amphibians from the chytrid fungus, which is already implicated in the extinction of 90 species [51].

- Major concerns about the use of gene drives are that they will escape control, entering non-target populations, jumping between species, and having unintended negative consequences. However, it is possible that the safest and most effective use of gene drives will instead be to use them in coordination with existing control techniques [11]. For example, to target a weed a gene drive that carries susceptibility to a herbicide might be released in an agricultural area. This drive carries little immediate cost, so may spread rapidly. The fitness cost will only become apparent when herbicides are actually deployed in the fields, and the controlled use limits these costs to the target areas. Even if the gene drive spread to wild populations of the weed species, or related non-pest species, the cost of well-designed herbicide susceptibility is likely to be low except where herbicides are deployed. One of the issues with gene drives designed to spread rapidly and exterminate the target organism is monetarisation, as a drive that rapidly eradicates the target species has not got a long-term income stream. Gene drives developed as part of a holistic pest control plan, where damage from the driver is dependent on the deployment of a second factor, might be safe and controllable, long term financially successful, and more acceptable to the public.
- The natural gene drives that have been discovered, and the synthetic drivers that have been constructed, are relatively direct in their action. They spread by converting genes, killing gametes that do not carry drive, shred rival chromosomes, and use other rather brute force approaches. However, it is likely that many more subtle possibilities for gene drive exist. In fact, many of them may already exist in nature, but have not yet been discovered because researchers are not looking for them, or interpreting them as drive. A fascinating example occurs in fire ants (*Solenopsis invicta*). A gene driver, the *Gp-9* locus, within a large inversion, has behavioural effects on drive-carrying workers that result in a transmission advantage for the locus – by selective elimination of non-carrier queens and tolerance of multiple carrier queens within the colony [52, 53]. It has ecological consequences, as fire ants are invasive in North America, and invasion success is associated with increased queen number [54]. There are likely to be other non-reproductive gene drives, perhaps driven by parental care biases, or siblicide, that have yet to be discovered, or thought of.

Collectively, the contributions of this Special Feature demonstrate the tremendous potential of gene drive systems, but also highlights several outstanding knowledge gaps. In particular, the wider ethical and societal implications of harnessing and unleashing the power of selfish genes in natural populations are still only in the early stages of being addressed.

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